Animal Feed Science and Technology 151 (2009) 330–336

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

Toughness, particle size and chemical composition of meadow fescue (*Festuca pratensis* Hud.) herbage as affected by time of day

P. Gregorini [a,*], K.J. Soder [a], M.A. Sanderson [a], G.R. Ziegler [b]

[a] *USDA/Agricultural Research Service, Pasture Systems, and Watershed Management Research Unit, Building 3702, Curtin Road, University Park, PA 16802-3702, USA*
[b] *Center for Food Manufacturing, The Pennsylvania State University, 341 Food Science Building, University Park, PA 16802, USA*

ARTICLE INFO

Article history:
Received 15 June 2008
Received in revised form 12 February 2009
Accepted 20 February 2009

Keywords:
Herbage chemical composition
Toughness
Grazing behaviour

ABSTRACT

While herbage chemical composition varies diurnally, it is not known if this variation affects herbage biomechanical properties. The objective of this study was to evaluate changes in herbage toughness and particle size in relation to diurnal fluctuations of herbage chemical composition. Vegetative (i.e, tillers with three fully expanded leaves) micro-swards of *Festuca pratensis* Hud. were sampled at four times of the day being: 06:50 (sunrise), 11:10, 15:30 and 19:25 h. Cut herbage was analysed for dry matter (DM), crude protein (CP), total non-structural carbohydrates (TNC), neutral and acid detergent fibre (aNDF, ADF), *in vitro* true digestibility (IVTD), toughness and particle size. The experiment was repeated on two consecutive days. Diurnal variation of temperature, relative humidity and photosynthetic radiation were recorded every 5 min with an automated weather station. To characterize the relationship between dependent variables, Pearson correlations were performed. From 06:50 to 19:25 h, DM, TNC and the proportion of small particles (<1 mm) increased ($P<0.05$), CP, aNDF, ADF, toughness and proportion of large particles (>4.75 mm) decreased ($P<0.05$) while IVTD remained constant ($P>0.05$). Toughness was negatively correlated ($P<0.05$) with DM and TNC concentrations, and positively correlated ($P<0.05$) with proportions of aNDF and

Abbreviations: ADF, acid detergent fiber; aNDF, neutral detergent fiber; CP, crude protein; N, Newton; TNC, total non-structural carbohydrates; DM, dry matter; IVTD, *in vitro* true digestibility.

* Corresponding author. Present address: DairyNZ Ltd., Private Bag 3221, Corner Ruakura and Morrisville Roads Hamilton, New Zealand. Tel.: +64 7 858 3787; fax: +64 7 858 3751.
E-mail address: Pablo.Gregorini@dairynz.co.nz (P. Gregorini).

0377-8401/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.anifeedsci.2009.02.004

ADF. The proportion of small particles was positively correlated (P<0.05) with DM and TNC concentrations, and negatively correlated (P<0.05) with proportions of aNDF, ADF and toughness. Results suggest an effect of time of day on herbage toughness and particle size as a function of increases in DM concentration, and reductions of aNDF and ADF concentrations, due to an increase in TNC. Diurnal fluctuations in chemical composition of herbage not only result in differential nutrient supply to grazing ruminants during the day, but also in temporal fluctuations in herbage biomechanical features.

1. Introduction

Several studies have shown increases in dry matter (DM) and total non-structural carbohydrate (TNC) concentrations of herbage during the day because of moisture losses and accumulation of photosynthates (Mayland et al., 2003; Griggs et al., 2005). Increased TNC dilutes neutral and acid detergent fibres (NDF, ADF) and crude protein (CP) concentrations (Delagarde et al., 2000).

Toughness has been described as the resistance to 'cracking' of plant material (Atkins and Mai, 1985; Lucas et al., 1995), and the quantity and association of cellulose fibres, hemicellulose and lignin in plant cell walls, to some degree, determine toughness (Grenet and Belse, 1991). Thus, concentrations of NDF and ADF may reflect the toughness of plant material and chewing effectiveness during ingestion and rumination (Perez-Barberia and Gordon, 1998).

Ingestive chewing is critical for grazing ruminants because it starts the particle comminution process, as well as facilitates bolus formation, cell content release, degradation and particle passage from the rumen (Chilibroste et al., 2008). Consequently, diurnal changes in herbage chemical composition can result in substantial differences in nutrients supplied by pasture throughout the day (Mayland et al., 2005; Gregorini et al., 2008a), and may expose grazing ruminants to diurnal fluctuations of herbage biomechanics.

The objective of this experiment was to quantify changes in 'toughness' and particle size of meadow fescue (*Festuca pratensis* Hud.) herbage in relation to diurnal fluctuations of herbage chemical composition.

2. Materials and methods

2.1. Site and experimental setup

The experiment was conducted at the USDA-ARS Pasture Systems and Watershed Management Research Unit in University Park, PA, USA. In January 2007, monoculture micro-swards (Orr et al., 2005) of meadow fescue were established in 22 plastic boxes (79 cm × 47 cm × 11.5 cm; 4.5 kg when empty). All boxes had 6 mm drainage holes drilled in the bottom spaced at 10 cm in four rows of seven (*i.e.*, 28 total holes). Each box was filled with 40–45 kg of a potting medium (Scott's Sierra Horticultural Products Co., Marysville, OH, USA). Seeding rate was 500 seeds/m^2, based on the germination rate previously determined. The micro-swards were established and grown in the greenhouse for 14 weeks, and then placed outdoors in May. Micro-swards were cut at 6 cm stubble height about every 21 days to maintain vegetative (*i.e.*, three fully expanded leaves) tillers. Micro-swards were watered regularly to maintain soil moisture at field capacity and fertilized after each 21 days cutting interval with N, P, and K at 1.79, 3.58 and 1.79 g/m^2, respectively.

2.2. Micro-sward sampling and measurements

Sampling was on consecutive sunny, or a few clouds, days (*i.e.*, September 12 and 13, 2007) to create one set of 11 micro-swards per day. Micro-swards were sampled four times per day being: 06:50 (sunrise), 11:10, 15:30 and 19:25 h (sunset). A grid was set in each micro-sward and samples were collected from randomly selected grid points within each micro-sward. Photosynthetic active radiation (LI-COR, LI190SB Quantum Sensor, Logan, UT, USA), temperature and relative humidity (Vaisala, HMP35C, Logan, UT USA) were measured every 5 min each day with an automated weather station.

2.2.1. Toughness

Three leaf blades from different tillers were randomly harvested from each micro-sward at each time of the day. These blades corresponded to the second fully expanded leaf. Blades were immediately put into nylon bags (20 cm × 15 cm), placed in an ice chest and analysed for force in compression and fracture within 30 min of harvested. Fracture has been utilized by Wright and Illius (1995) and Aranwela and Sanson (1999) as a measure of toughness. The values of toughness for each blade was the mean force in compression of three equal-length portions (*i.e.*, top, middle, bottom) of the blades using in a TA-XT2i texture analyser (Texture Technologies Corp., Scarsdale, NY, USA). Cutting blades (sharpened, 0.6 mm thick) passed through the leaf material at a rate of 10 mm/s and break return sensitivity was 0.9 N. Stable Micro Systems and Texture Expert Exceed™ software (Stable Micro Systems, Ltd., Texture Technologies Corp., Surrey, England, UK) were used to calculate total work in Newton. This value was divided by the cross sectional area (leaf-blade width × thickness) at the breaking point for the calculation of toughness (*i.e.*, N/mm^2) (Wright and Illius, 1995; MacAdam and Mayland, 2003). Width and thickness of the leaf-blade were measured with a digital calliper (14-648-17, Control Co. West Englewood, TX, USA).

2.2.2. Particle size

The effect of time of day on particle size was determined similarly to the particle size reduction index technique of Casler et al. (1996). At each time of the day, approximately 10 g (DM basis) of meadow fescue were harvested from each micro-sward to a 6 cm stubble height to minimize pseudo-stems in the samples. Samples were immediately frozen in liquid nitrogen for further freeze-drying (Ultra 35 Super ES, Virtis, Gardiner, NY, USA) and analysis. A 4 g dry sub-sample from each sample was ball-milled in a hobby-sized rock polishing machine (Thumler A-R1, Tru-Square Metal Products, Auburn, WA, USA). The rubber cylinder was 125 mm in diameter and 110 mm deep, with a speed of 0.43 revolutions/s. One hundred stainless-steel ball bearings with 9.5 mm diameter and 100 ball bearings with a 12.7 mm diameter were included in the rubber cylinder. Samples were ball milled for 30 s (Casler et al., 1996). After ball-milling, contents of the rubber cylinder were removed and brushed onto a 4.75 mm screen (USA Standard Testing Sieve, Fisher Scientific Co, New York, NY, USA). This screen rested on the top of a 1 mm screen (USA Standard Testing Sieve, Fisher Scientific Co, New York, NY, USA). Sieving time was 60 s. After sieving, residues from each sieve were weighed and proportions from the initial sample weight (4 g DM) were calculated. The proportion of particles passing through the 1 mm screen is equivalent to the particle size reduction index calculated by Casler et al. (1996).

2.2.3. Meadow fescue chemical composition

The remaining 6 g of DM of the meadow fescue samples collected for particle size reduction rate were analysed for aNDF, ADF, nitrogen and TNC. Dry matter was determined by freeze-drying (Ultra 35 Super ES). The methods of Van Soest et al. (1991) were used in the analyses of aNDF and ADF with amylase and sodium sulphite used in the aNDF procedure and both aNDF and ADF are expressed inclusive of ash. Concentrations of nitrogen were determined by total Kjeldahl nitrogen, (official method 976.06 with 75 ml calibrated tubes and $CuSO_4/K_2SO_4$ catalyst; AOAC, 2000) and analysis on Quickchem 8000 Ion Analyser (Lachat Instruments, Milwaukee, WI, USA). The TNC content was determined by procedures of Burns et al. (2006). Determination of *in vitro* true digestibility (IVTD) was according to Goering and Van Soest (1970) with a 48-h rumen fluid digestion followed by post digestion aNDF analysis. Multi-layer polyethylene polyester cloth bags (F57 filter bag; ANKOM Technology Corp., Macedon, NY, USA) were utilized for incubation of meadow fescue samples for DM digestibility. Bags were rinsed with acetone and air-dried for 24 h before use. Ground samples (0.25 g/bag) were weighed into triplicate bags, heat sealed and placed in digestion jars. The incubation media and procedure were similar to that described by Robinson et al. (1999). At the end of the 48 h incubation, jars were removed from the chamber, the incubation solution was discarded, and the bags were rinsed four times with distilled water. Bags were placed in an ANKOM fibre (aNDF) analyser and boiled in ND solution for 75 min for *in* *vitro* true digestibility determination. Bags were removed, soaked in acetone for 3 min and oven-dried at 100 °C for 24 h. The IVTD was calculated as the difference between DM incubated and the residue after ND extraction.

2.3. Experimental design, treatments and statistical analyses

Data were analysed as a completely randomized design with the experimental unit being the micro-sward. Times of day (*i.e.*, 06:50, 11:10, 15:30 and 19:25 h) were considered as treatments. Data was analysed using random coefficient regression. REML was used to fit a mixed model including day and linear and quadratic effect of time of day as fixed effects and micro-sward and linear and quadratic effects within micro-sward as random effects. A value of $P<0.05$ was considered to be significant. To characterize the relationship between dependent variables, Pearson correlations were performed. GenStat 11.1 was used for all statistical analyses (Payne et al., 2008).

3. Results and discussion

As there was no treatment × day interactions for any variable analysed, averages over both sampling days are presented and variables are discussed in terms of treatment.

Dry matter concentrations of the herbage were relatively low (Table 1), which is likely related to the young vegetative stage of the meadow fescue sampled. In addition, leaf blades comprised nearly all of each sample because of the 6 cm stubble cut height. These conditions, the morning dew, the diurnal changes in temperature and relative humidity (Fig. 1), as well as the linear increase in TNC (Table 1) may explain the marked changes in DM from 06:50 to 19:25 h. The CP, ADF and aNDF concentrations decreased linearly from 06:50 to 19:25 h by 8.5%, 10.3%, and 5.1 %, respectively. Concentrations of ADF and aNDF were negatively correlated ($P<0.001$) with DM (−0.59 and −0.54, respectively) and TNC (−0.76 and −0.64, respectively). The TNC increased by 46.2% by 19:25 h *versus* 06:50 h. There was a quadratic effect of time of day for DM, CP and TNC. This shape of these diurnal fluctuations may be related to an increase in respiration and metabolic activity of the plants at dusk. Although there were intermittent clouds during day 2 (Fig. 1), the general pattern of photosynthetic active radiation did not differ among days. Therefore, the shape of the diurnal increase in TNC may be due to changes in photosynthetic active radiation and air temperature from 15:00 h onwards. These results are comparable to the diurnal fluctuations of TNC and fibres reported by Delagarde et al. (2000) and Gregorini et al. (2006a). Time of day did not affect IVTD, which is similar to the low variation in digestibility of organic matter reported by Delagarde et al. (2000). The lack of effect of time of day on IVTD may suggest that IVTD is a poor indicator of forage nutritive and feeding value since the forage structure and mechanical features are destroyed by the grinding required to prepare samples for IVTD determination, thereby removing any impact of ‘toughness’ on IVTD.

Table 1
Effect of time of day on herbage dry matter (g/kg fresh matter), chemical composition, *in vitro* true digestibility (g/kg DM), toughness (N/mm^2), and particle size ((g/kg DM) of herbage.

Time of day (h)	DM	CP	ADF	aNDF	TNC	IVTD	Toughness	Particle size*		
								L	*M*	*S*
06:50	78.5	172.1	313.3	507.4	123.3	897.9	1.82	250.0	727.0	17.5
11:10	110.5	166.4	300.7	486.4	148.4	903.0	1.40	225.1	747.5	22.5
15:30	137.6	155.5	293.0	478.1	176.8	903.4	1.15	150.0	800	60.0
19:25	150.2	157.5	281.2	481.6	180.3	905.9	1.11	117.5	827.5	50.0
SED	2.72	2.85	3.10	4.04	3.30	3.38	0.066	0.20	18.71	14.52
P linear	<0.001	<0.001	<0.001	<0.001	<0.001	0.249	<0.001	<0.001	<0.001	<0.01
P quadratic	<0.001	0.076	0.988	<0.001	<0.001	0.498	<0.001	0.640	0.616	0.873

DM, dry matter; CP, crude protein; ADF, acid detergent fibre; aNDF, neutral detergent fibre; TNC, total non-structural carbohydrates; IVTD, *in vitro* true digestibility of DM.

* Proportion of the 4 g DM sample; *L*, >4.75 mm; *M*, <4.75 > 1 mm; *S*, <1 mm.

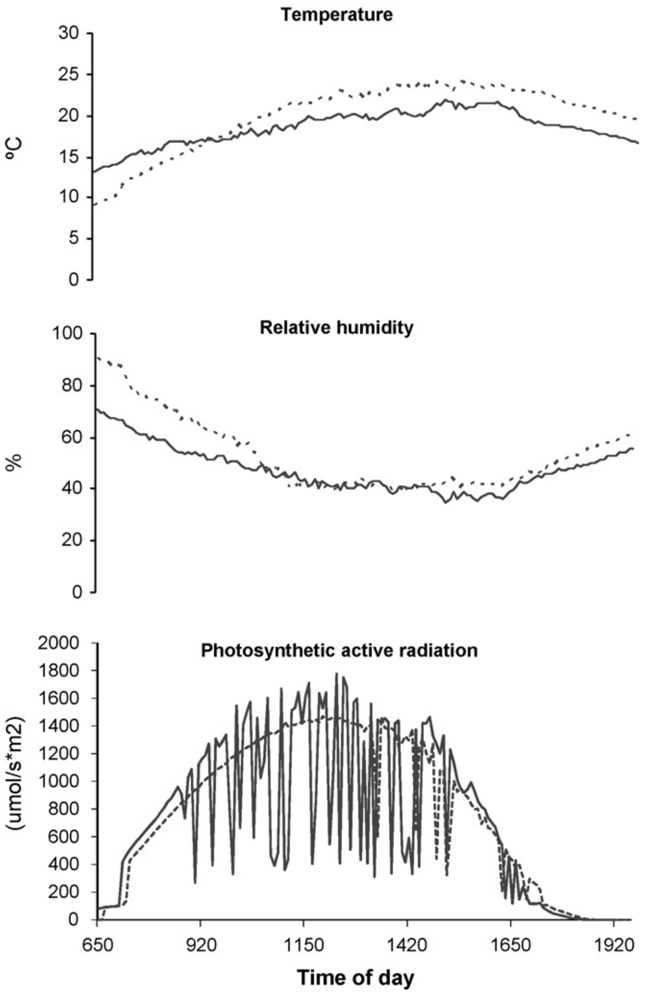

Fig. 1. Diurnal variation of weather conditions during the two measurement days. (Solid line, day 1; dashed line, day 2.)

'Toughness' decreased by 38.6% from 06:50 to 19:25 h (Table 1). In general, as materials dry they become more brittle (Vincent, 1983). Despite the low DM content of the meadow fescue analysed, the marked increase in DM content from 06:50 to 19:25 h affected 'toughness', which is supported by the negative correlation (−0.63; P<0.001) between DM and 'toughness', and it also agrees with Choong et al. (1992). 'Toughness' was also negatively correlated with TNC (−0.58; P<0.001) and positively correlated with ADF and aNDF (0.53 and 0.58, respectively; P<0.001). These results concur with the positive correlation between the 'toughness' and fibre content of leaves of tropical trees (Choong et al., 1992). Our results also agree with previous research that identified correlations between fibre (*i.e.*, NDF and ADF) content and maximum force, work to fracture and 'toughness' of temperate grasses leaves (Wright and Illius, 1995). The effect of time of day on particle size reduction (Table 1) followed the same trend as 'toughness', similar to fibres and opposite to DM and TNC. From 06:50 to 19:25 h there was a linear decrease in the proportion of large particles (*i.e.*, >4.75 mm) and a linear increase in the proportion of medium-sized particles (i.e, <4.75 > 1 mm). Casler et al. (1996) argued that the proportion of small particles (*i.e.*, <1 mm) could be related to ease of comminution of grasses, whereas Perez-Barberia and Gordon (1998) suggested that comminution is a function of 'toughness', which may relate to the proportions of NDF and ADF in the herbage. In the present study, the proportion of small particles was negatively correlated with 'toughness', aNDF and ADF (−0.50 and −0.53, respectively; $P<0.001$), and positively correlated with TNC (0.58; $P<0.001$). These correlations, and the lack of effect of time of day on IVTD, matches the results of Casler et al. (1996), who reported negative correlation between proportion of small particles and fibre concentrations, and a lack of association between the particle size reduction index and fibre digestibility. These results suggest that grass leaves consumed by grazing ruminants during the afternoon and evening would be more easily comminuted than leaves consumed in the morning.

Ingestive comminution is a critical process for grazing ruminants because it facilitates microbial colonization of feed particles (Chilibroste et al., 2008), thereby affecting degradation and particle passage through the rumen. According to our results, meadow fescue herbage consumed at dusk may be more easily swallowed, and passed through the rumrn, as well as 'packed' in the rumen (Chilibroste et al., 2005). This premise is supported by results of Gregorini et al. (2008b), who reported higher particle passage rates when beef heifers were stimulated to graze during dusk hours. Ingestive comminution is energetically the most expensive task during grazing (Wright and Illius, 1995; Di Marco and Aello, 1998). Grazing ruminants prefer leaves (MacAdam and Mayland, 2003) and graze longer and eat more during dusk (Gibb et al., 1998; Gregorini et al., 2006b). Taken in the context of our results, we suggest that cattle preference to graze at dusk is partly related to diurnal fluctuations of herbage leaves toughness. Grazing at dusk would be energetically more profitable.

4. Conclusions

The diurnal increment of herbage DM, TNC, and the concomitant dilution of aNDF and ADF concentration diminish meadow fescue toughness and increase of particle size reduction from dawn to dusk. During the afternoon and evening, grass leaves would be more easily comminuted than leaves grazed in the morning, which may help to explain diurnal behavioural patterns of grazing ruminants.

Acknowledgements

The authors thank Melissa Rubano, USDA-ARS, and Dr. Rajesh Bhosale, Center of Food Manufacturing, for laboratory expertise and time contributed to conducting this study. The authors also thank Drs. Pablo Soca, Michael H. Wade and Stacey A. Gunter for their critical review and comment on the manuscript.

References

A.O.A.C., 2000. Official Methods of Analysis of the Association of Official Analytical Chemists, 17th ed. Washington, DC, USA. p. 1018.

Aranwela, N., Sanson, J.R., 1999. Methods of assessing leaf fracture properties. New Phytol. 144, 369–383.

Atkins, A.G., Mai, Y.W., 1985. Elastic and Plastic Fracture, 1st ed. Ellis Horwood, Ltd., West Sussex, England, UK, p. 439.

Burns, J.C., Fisher, D.S., Rottinghouse, G.E., 2006. Grazing influences on mass, nutritive value, and persistence of stockpiled Jessup tall fescue without and with novel and wild-type fungal endophytes. Crop Sci. 46, 1898–1912.

Casler, M.D., Scheider, D.K., Combs, D.K., 1996. Development and application of a selection criterion for particle size breakdown of smooth bromegrass leaves. Anim. Feed Sci. Technol. 61, 57–61.

Chilibroste, P., Gibb, M.J., Tamminga, S., 2005. Pasture characteristics and animal performance. In: France, J., Forbes, M., Dijkstra, J. (Eds.), Quantitative Aspects of Ruminant Digestion and Metabolism. CAB International, Wallingford, Oxon, England, UK, pp. 681–706.

Chilibroste, P., Dijkstra, J., Robinson, P.H., Tamminga, S., 2008. A simulation model "CTR Dairy" to predict the supply of nutrients in dairy cows managed under discontinuous feeding patterns. Anim. Feed Sci. Technol. 143, 148–173.

Choong, M.F., Lucas, P.W., Ong, J.S.Y., Pereira, B., Tan, H.T.W., Turner, I.M., 1992. Leaf fracture toughness and sclerophylly: their correlations and ecological implications. New Phytol. 121, 597–610.

Delagarde, R.J., Peyraud, J.L., Delaby, L., Faverdin, P., 2000. Vertical distribution of biomass, chemical composition and pepsin-cellulase digestibility in a perennial ryegrass sward: interaction with month and year, re-growth age and time of day. Anim. Feed Sci. Technol. 84, 49–68.

Di Marco, O.E., Aello, M.S., 1998. Energy cost of cattle walking on the level and on a gradient. J. Range Manage. 51, 9–13.

Gibb, M.J., Huckle, C.A., Nuthall, R., 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass Forage Sci. 53, 41–46.

Goering, H.K., Van Soest, P.J., 1970. Forage fibre analyses (apparatus, reagents, procedures and some applications). Agricultural Handbook no. 379. ARS-USDA, Washington, DC, USA.

Gregorini, P., Eirin, M., Refi, R., Ursino, M., Ansin, O., Gunter, S.A., 2006a. Timing of herbage allocation. Effect on beef heifers daily grazing pattern and performance. J. Anim. Sci. 84, 1943–1950.
Gregorini, P., Tamminga, S., Gunter, S.A., 2006b. Review: behavior and daily grazing patterns of cattle. Prof. Anim. Scientist 22, 201–209.
Gregorini, P., Gunter, S.A., Beck, P.A., Soder, K.J., Tamminga, S., 2008a. Review: the interaction of diurnal grazing pattern, ruminal metabolism, nutrient supply and management in cattle. Prof. Anim. Scientist 24, 308–318.
Gregorini, P., Gunter, S.A., Beck, P.A., 2008b. Matching plant and animal processes to alter nutrient supply in strip grazed cattle: Timing of herbage and fasting allocation. J. Anim. Sci. 86, 1006–1020.
Grenet, E., Belse, J.M., 1991. Microbes [of rumen] and fiber degradation. In: Jouany, J. (Ed.), Rumen microbial metabolism and ruminant digestion, Institut National de la Recherche Agronomique, Paris, France INRA, 1991. ISBN: 2-7380-0345-1, pp. 107–129.
Griggs, T.C., MacAdam, J.W., Mayland, H.F., Burns, J.C., 2005. Non-structural carbohydrate and digestibility patterns in orchard-grass swards during daily defoliation sequences initiated in evening and morning. Crop Sci. 45, 1295–1304.
Lucas, P.W., Darvell, D.W., Lee, P.K.D., Yuen, T.D.B., Choong, M.F., 1995. The toughness of plant cell walls. Phil. Trans.: Biol. Sci. 348, 362–372.
MacAdam, J.W., Mayland, H.F., 2003. The relationship of leaf strength to cattle preference in tall fescue cultivars. Agron. J. 95, 414–419.
Mayland, H.F., MacAdam, J.W., Shewmaker, G.E., Chatteron, N.J., 2003. The diurnal cycling of sugars in grasses impact strip-graze management plans. In: Proceedings of the Second National Conference of Grazing Lands, Dec. 7–10, 2003, Nashville, TN, USA, pp. 7–10.
Mayland, H., Gregorini, P., Mertens, D.R., Taylor, J.B., Burns, J.C., Fisher, D.S., Ciavarella, T., Smith, K., Shewmaker, G., Griggs, T., 2005. Diurnal changes in forage quality and their effects on animal preference, intake, and performance. In: Proceedings of the 35th California Alfalfa & Forage Symposium, December 12–14, 2005, Visalia, CA, USA, pp. 223–230.
Orr, R.J., Young, K.L., Cook, J.E., Champion, R.A., 2005. Development of a micro-sward technique for determining intake characteristics of perennial ryegrass varieties. Euphytica 141, 65–73.
Payne, R.W., Harding, S.A., Murray, D.A., Soutar, D.M., Baird, D.B., Glaser, A.I., Channing, I.C, Welham, S.J., Gilmour, A.R., Thompson, R., Webster, R., 2008. GenStat Release 11 Reference Manual, Part 2 Directives. VSN International, Hemel Hempstead, England.
Perez-Barberia, F.J., Gordon, I.J., 1998. Factors affecting food comminution during chewing in ruminants: a review. Biol. J. Linnean Soc. 63, 233–256.
Robinson, P.H., Campbell Mathews, M., Fadel, J.G., 1999. Influence of storage time and temperature on in vitro digestion of neutral detergent fibre at 48 h, and comparison to 48 h in sacco neutral detergent fibre digestion. Anim. Feed Sci. Technol. 80, 257–266.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Vincent, J.F.V., 1983. The influence of water content on the stiffness and fracture properties of grass leaves. Grass Forage Sci. 38, 107–114.
Wright, W., Illius, A.W., 1995. A comparative study of the fracture properties of five grasses. Funct. Ecol. 9, 269–278.